International Journal of Agricultural Science and Research (IJASR)
ISSN(P): 2250-0057; ISSN(E): 2321-0087
Vol. 4, Issue 1, Feb 2014, 1-6
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EFFICACY OF SOME MEDICINAL PLANT EXTRACTS AGAINST *ASPERGILLUS NIGER* OF WHEAT SEEDS

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ABSTRACT

Four medicinal aqueous leaf extracts, namely *Cinnamomum virum, Anisomeles malabarica, Azadirachta indica* and aqueous seed extract of *Nigella sativa* were evaluated under in vitro conditions against the radial growth of black mold (*Aspergillus niger*), which isolated from the wheat seeds. A total of five treatments, replicated three were taken up in complete randomized design. Each leaves and seed extracts were made at different concentrations *viz*. 2%, 4%, 6%, 8% and 10% against *Aspergillus niger*. After 6 days of incubation results revealed that leaves and seed extracts inhibited the radial growth of test pathogen and the effect increased with increased the concentration % on compared with control. Among the medicinal plants, *Nigella sativa* seed extract gave the significantly inhibition percentage of *Aspergillus niger* at all concentrations.

KEYWORDS: *Aspergillus niger*, Antifungal, *Cinnamomum virum, Anisomeles malabarica, Azadirachta indica* and *Nigella sativa*

INTRODUCTION

Wheat (*Triticum aestivum* L.) is one of the most important cereal crop of the world. It belongs to family Graminae (Poaceae). Wheat is staple food crop for about one billion people in as many as 43 countries and provides about 20% of the total food calories [7]. Seed health plays an important role for successful cultivation and yield exploitation of a crop species. Among various factors that affect seed health, the most important are the seed borne fungi that not only lower seed germination, but also reduce seed vigor resulting in low yield. Healthy seed plays an important role not only for successful cultivation but also for increasing yield of crop. Seeds are regarded as highly effective means for transporting plant pathogens over long distances. Numerous examples exist in agriculture literature for the international spread of plant diseases as a result of the importation of seeds that were infected or contaminated with pathogens [3].

A significant portion of the agricultural produce in the country and the world over become unfit for human consumption due to mycotoxins contamination of grains, especially those produced by species of *Aspergillus* [10]. More than 25% of the world cereals are contaminated with known mycotoxins and more than 300 fungal metabolites are reported to be toxic to man and animals [11]. Seed fungi have especially species of *Aspergillus* spp, *Diplodia* spp, *Penicillium* spp, *Fusarium* spp, *Trichoderma* spp and a number of *Phycomycetes* affect the seed. Some fungi have capabilities of producing toxins [9]. Medicinal plants are a source of great economic value all over the world. Nature has bestowed on is a very rich botanical wealth and a large number of diverse types of plants grow in different parts of the country**.** Traditional and folklore medicines play important role in health services around the globe. About three quarter of the world's population relies on plants and plant extracts for health care**.**

Traditionally the fungal diseases of plants are controlled by using synthetic fungicides. The uses of fungicides were not only expensive but also hazardous to the environment. On the other hand, the indiscriminate use of pesticides may result into development of resistance in the pathogens. To overcome these problems, some alternative control methods must be employed. The antifungal action of plant extracts has gained much attention. Now days, plants are being used against many plant pathogenic fungi. The plants serve as ecofriendly and economic biocontrol agents. Thus, there is a need for the development of alternative disease control materials that are both effective in plant disease control and at the same time environmentally friendly. It has been several scientists reported that farmers and local inhabitants especially in developing countries frequently use plants with medicinal properties to alleviate certain illnesses and some times to protect their farm produce from spoilage [2].

MATERIALS AND METHODS

The experiment was conducted under laboratory conditions in the Department of Plant protection, Sam Higginbottom Institute of Agriculture, Technology & Sciences, Allahabad (India) during the year 2013.

Isolation of Fungi from Infected Wheat Seeds

Infected wheat seeds were collected from different stores and markets from Allahabad city U.P. India. The infected wheat seeds were surface sterilized with 0.1% mercuric chloride solution, thrice rinsed with sterilized distilled water and aseptically transferred 5 seeds per plate in to containing melted lukewarm PDA medium. After solidifying petri plates were kept inverted position in incubator at 25 ± 1^{0C} for 6 days. On 4rd day brownish blackish growth of mycelium was observed in Petri plates. Some part of colony was taken and slide prepared. After that slide was observed under the microscope. Conidiophore was long, erect and arises from a foot cell. It forms a terminal swollen vesicle and conidia produce as a form of chain.

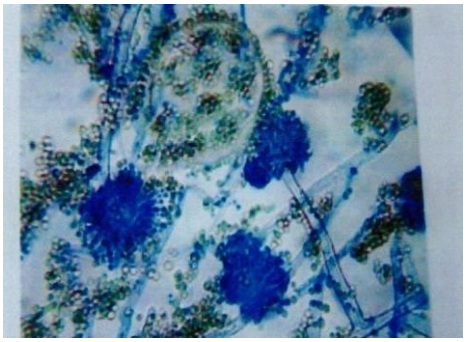

Figure 1: Conidiophore and Conidia *Aspergillus niger*

Figure 2: Pure Culture of *Aspergillus niger* of in PDA Slant

On the basis of microscopic observation the fungus was identified as *Aspergillus niger* (Figure 1) causative agent of black mold of wheat seed [8, 6].

Selection of Different Extract Used in the Test Experiments

Some medicinal plants *viz*. *Cinnamoum verum* (Cinnamon), *Anisomeles malabarica* (Malabar Catmint), *Azadirachta indica* (Neem) and *Niggella sativa* (Black seed) were tested against *Aspergillus niger*.

Preparation of Plant Extracts

The plant leaves of (*Anisomeles malabarica, Azadirachta indica, Cinnnamomum verum*) were washed with distelled water and dried in shade with (*Nigella sativa*) seeds. They were then finally grinded to get powder. Fifty grams of

each plant extract in powder form was homogenized by laboratory blender in 200 ml sterile distilled water for 10 min , and then left in dark glass Bottles for 72 h for complete extraction. The extracts were filtered through thin cheesecloth sheets. The final extracts were collected separately in other dark glass bottles and exposed to 60℃ in water bath for 30 min. The collected extracts were then stored in a refrigerator at 5℃ ntil needed. The plant extracts were added to conical flasks containing sterilized PDA before solidification to obtain the proposed concentrations of 2%, 4%, 6%, 8% and 10 % (v/v). 20 ml of amended media were poured into 9 cm diameter petri dishes, and another set of untreated PDA plates was used as control. For each treatment was replicated at three times (plates). All plates were inoculated individually with 0.5 cm diameter discs of the tested fungal cultures and then incubated in the incubator at 25±2℃ for 3 days , until the control plates reached approximately full growth [1]. The parameters were observed *viz*. comparison between medicinal plants aqueous extracts on the radial growth of *Aspergillus niger* at 2%, 4%, 6%, 8% and 10% concentrations at 6 days after incubation. Percentage inhibition of mycelial growth by the plant extracts was calculated using the formula. [12].

$$\text{Percent inhibition} = \frac{\text{Control} - \text{Treatment}}{\text{Control}} \text{x100}$$

RESULTS AND DISCUSSIONS

Results of interactive effect of some medicinal plant extracts against growth inhibition (%) of *Aspergillus niger* at different concentrations are presented in Table, Figure and Plate: 1.

Results revealed that significant difference among all the treatments. The leaf extracts of *Azadirachta indica*, *Cinnamomum verum* and aqueous seed extract of *Nigella sativa* inhibited the radial growth of *Aspergillus niger* on PDA medium after 6 days of incubation at different concentrations as compared with the leaf extract of *Anisomeles malabarica*. The treatment T_4 -10% *Nigella sativa* seed extract (97.30%) was significantly reduced the growth of *Aspergillus niger* followed by T_3 -10% *Azadirachta indica* leaf extract (72.60%), T_1 -10 *Cinnamomum verum* leaf extract (68.50%) and T_2 -10% *Anisomeles malabarica* extract (43.80%). Overall the treatments were found effective against *Aspergillus niger* as compared with control.

Table 1: Comparision between Plant Aqueous Extracts of Different Concentrations on Growth Inhibition (%) of *Aspergillus niger* at 6 Days after the Incubation

Treatments	2%	4%	6%	8%	10%	Mean
T_1-*Cinnamoum verum*	15.00	28.70	42.50	52.00	68.50	41.34
T_2-*Anisomeles malabarica*	13.70	20.50	27.40	34.20	43.80	27.92
T_3-*Azadirachta indica*	17.80	27.40	43.80	58.90	72.60	44.10
T_4-*Nigella sativa*	93.20	96.60	96.60	96.60	97.30	96.06
T_0-Control	0%					

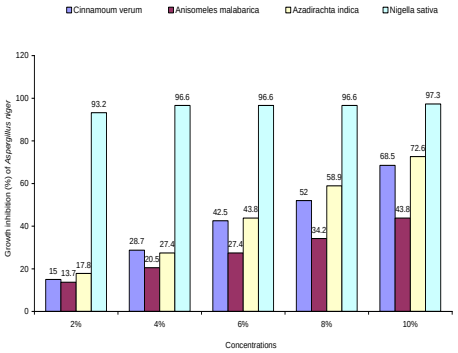

Figure 3: Comparision between Plant Extracts of Different Concentrations on Growth Inhibition (%) of *Aspergillus niger*

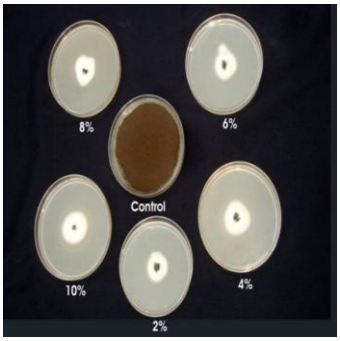

Figure 4: Effect of *Nigella sativa* at Different Concentrations on the Growth of *Aspergillus niger* at 6 Days after Incubation

DISCUSSIONS

It was evident from the results "In vitro" experiments that the growth of *Aspergillus niger* was significantly inhibited by these plant leaf and seed extracts. Of these plant extracts *Nigella sativa* seed extract was found more effective and showed the maximum inhibition of *Aspergillus niger* (97.30%). Similar observation was reported by [5] that *Nigella sativa* is active principles might have useful action against *Aspergillus niger*. [4] reported that effect of Neem (*Azadirachta indica*) leaves and seeds extract on the growth of six of the plant disease causing fungi.

ACKNOWLEDGEMENTS

The authors wish to thank Department of Plant Protection, Sam Higginbottom Institute of Agriculture, Technology & Sciences (Deemed-to-be-University) for the Scientific Support.

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